Supplementary Information

for

The PX Motif of DNA Binds Specifically to *Escherichia coli* DNA Polymerase I

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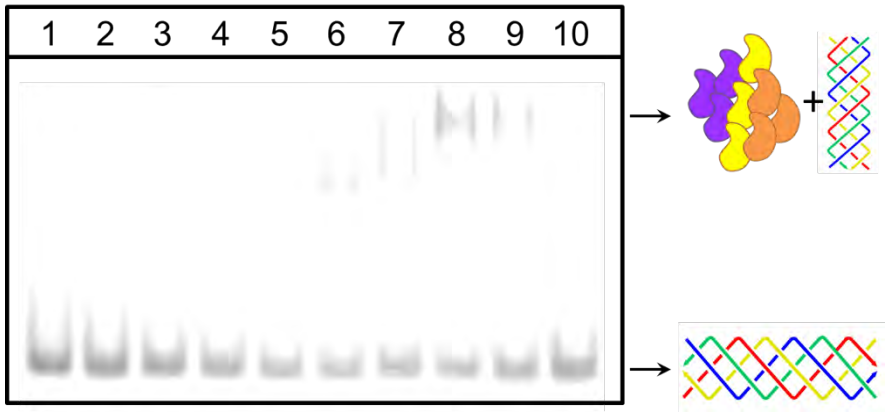


Figure S1. *PX-binding fractions collected from Q-sepharose columns.* The figure shows a non-denaturing gel of the different proteins fractions collected from Q-sepharose column (lanes 1-10) that bind to the PX complex shown in Figure 1. Lanes 6-9 show a gel shift indicating PX-binding of the specific protein fractions.

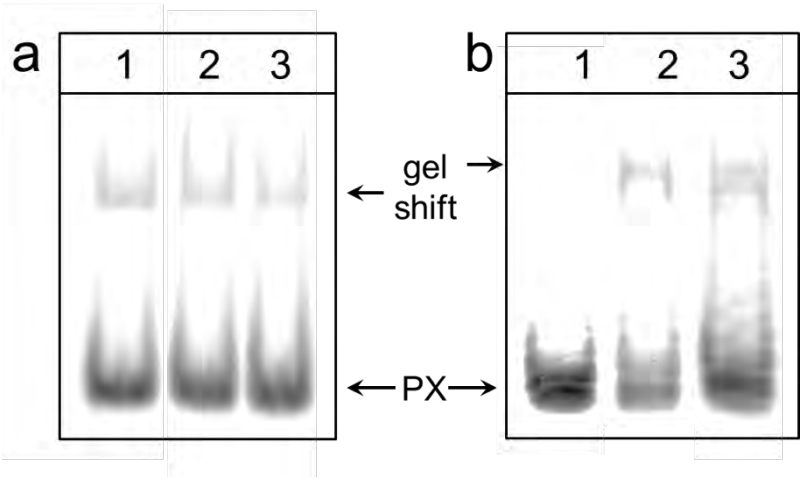


Figure S2. *PX binding of protein fractions in the presence of duplex competitors.* The protein fractions that bind to PX were isolated and analyzed on a non-denaturing gel for structure specific binding of the protein fractions to PX-DNA. (a) Lane 1 contains the PX complex and a duplex competitor poly(dI-dC), lane 2 contains the PX complex (PXC), 10X concentrated duplex competitors DC1 and DC2, lane 3 contains PXC, poly(dI-dC) and 10X DC1 and DC2. (b) A non-denaturing gel with higher concentration of duplex competitors. Lane 1 contains only PXC, lane 2 contains PXC and poly(dI-dC) and lane 3 contains PXC, poly(dI-dC) and 50X DC1 and DC2. In both (a) and (b) there is no difference in the gel shift for the lanes containing the duplex competitors DC1 and DC2 (same sequence as PXC) in the presence or absence of the common duplex competitor poly(dI-dC). This shows that the binding of protein fractions to DNA is structure-specific and not sequence-specific.

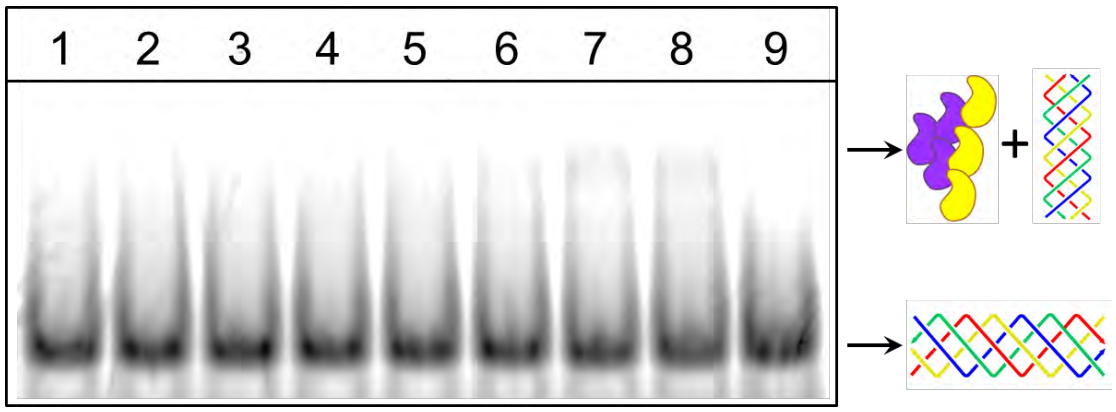


Figure S3. *PX-binding fractions collected from DNA cellulose columns.* The figure shows a non-denaturing gel of the different proteins fractions collected from DNA cellulose column according to the salt concentration (lanes 1-9) with PXC. Lanes 7 and 8 show a gel shift indicating binding of these protein fractions with PXC. These fractions were collected and used for purification through size columns.

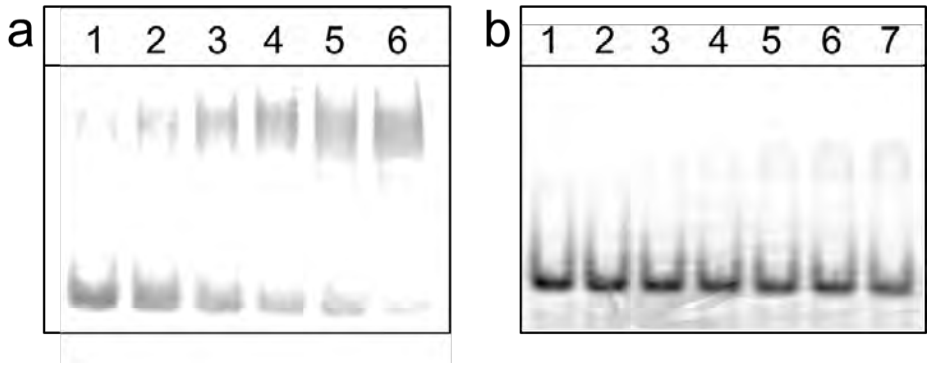


Figure S4. *Binding titration of protein fractions with PXC.* (a) Titration of the concentrated protein fractions collected from Q-sepharose column with PXC. Lanes 1 to 6 contain PXC binding with 1ul, 2ul, 3ul, 4ul, 5ul, and 6ul of the protein fractions. (b) Titration of the concentrated protein fractions collected from DNA cellulose column with PXC. Lane 1 contains only PXC, lanes 2-7 contain PXC with 1ul, 2ul, 3ul, 4ul, 5ul and 6ul of the protein fractions. In both cases, binding increases with higher amounts of the protein fraction.

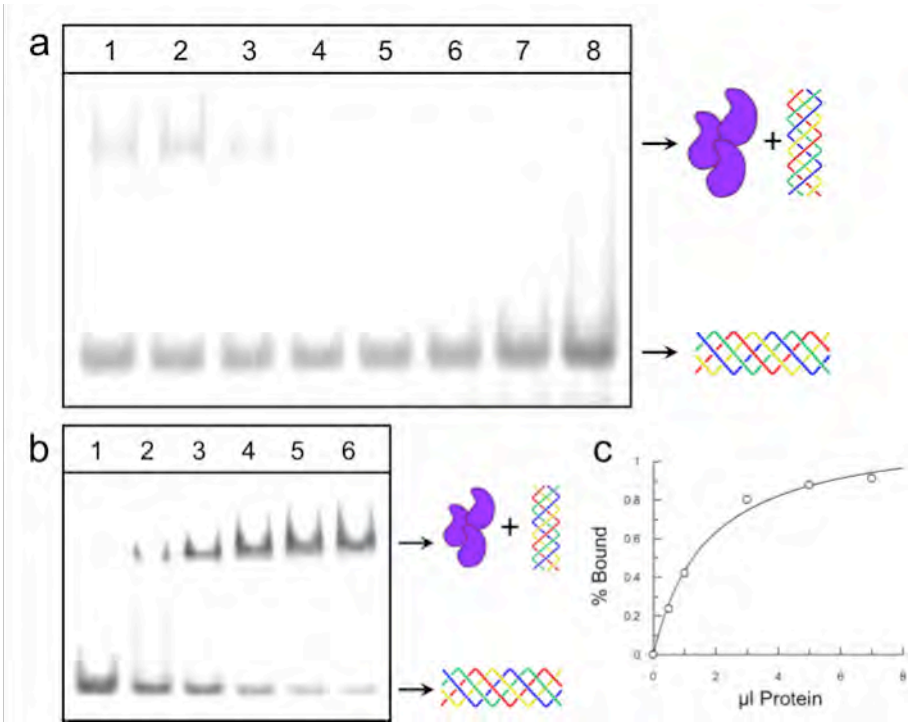


Figure S5. *PX-binding fractions collected from size columns.* (a) Binding results of different fractions collected from size column. Lanes 1-8 contain PXC with separated fractions. Gel shift in lanes 1-3 indicate PX-binding by these protein fractions. (b) Binding titration of PXC (lane 1) with different amount of the fractions from lanes 3-5 in (a). Lanes 2-6 have PXC with 1ul, 2ul, 3ul, 4ul and 5ul of protein fractions. (c) Binding curve using the data from (b) showing the binding of PXC with proteins with increasing quantities of protein fractions added

Figure S6. An SDS-PAGE gel for the final purified fraction collected from size column.

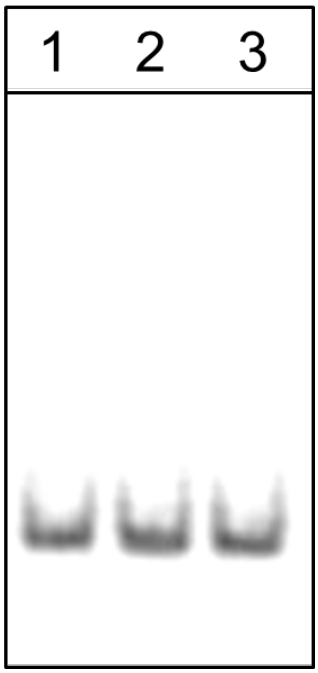


Figure S7. *PX binding results with ParC.* Lane 1: PX only. Lane 2: PX with 1ul 130mg/ml ParC. Lane 3: PX with 7ul 130mg/m

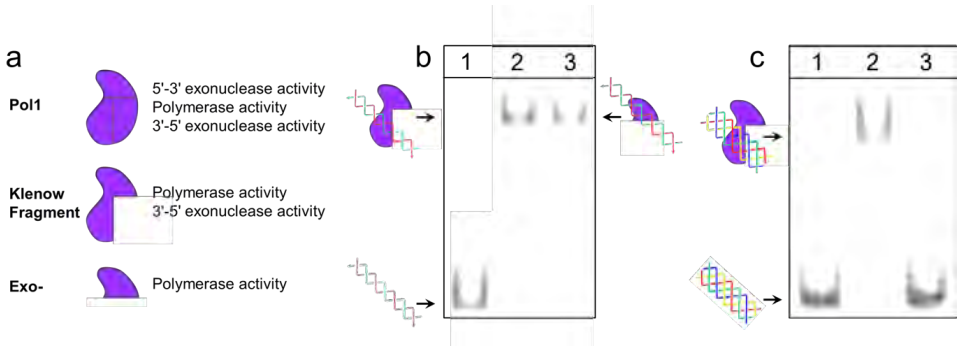


Figure S8. *Duplex and PX complex binding with Klenow and Exo- proteins.* (a) Cartoons of Pol I, Klenow fragment and the Exo- portion. (b) Duplex binding results with Klenow and Exo-. Lane 1 contains the only the duplex and lanes 2 and 3 contain the duplex with Klenow and Exo- respectively. (c) PXC binding results with Klenow and Exo-. Lane 1: PXC only. Lane 2: PXC and Klenow. Lane 3: PXC and Exo-.

Figure S9:

Sequences of DPE controls strands:

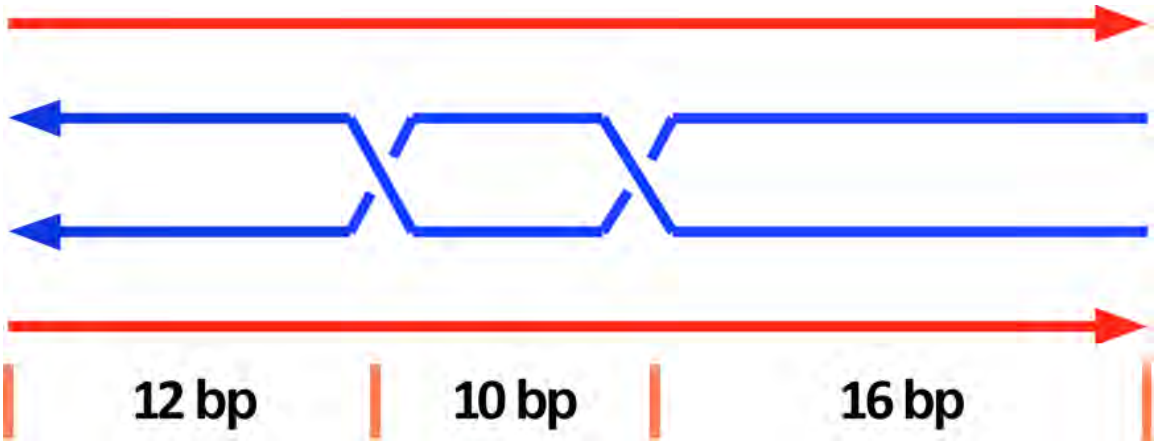
DX1: AGTGATTCGGTGCCTACACATATCTGTTGCGACACCAC

DX2: GTGGTGTCGCAACAGACACAATACTTCACCGAATCACT

DX3: ACTAGATCATCAATGCTATGTGTAGGGTTAGACCTGAG

DX4: CTCAGGTCTAACAAGTATTGTGGCATTGATGATCTAGT

The schematic drawing of DPE control molecule:



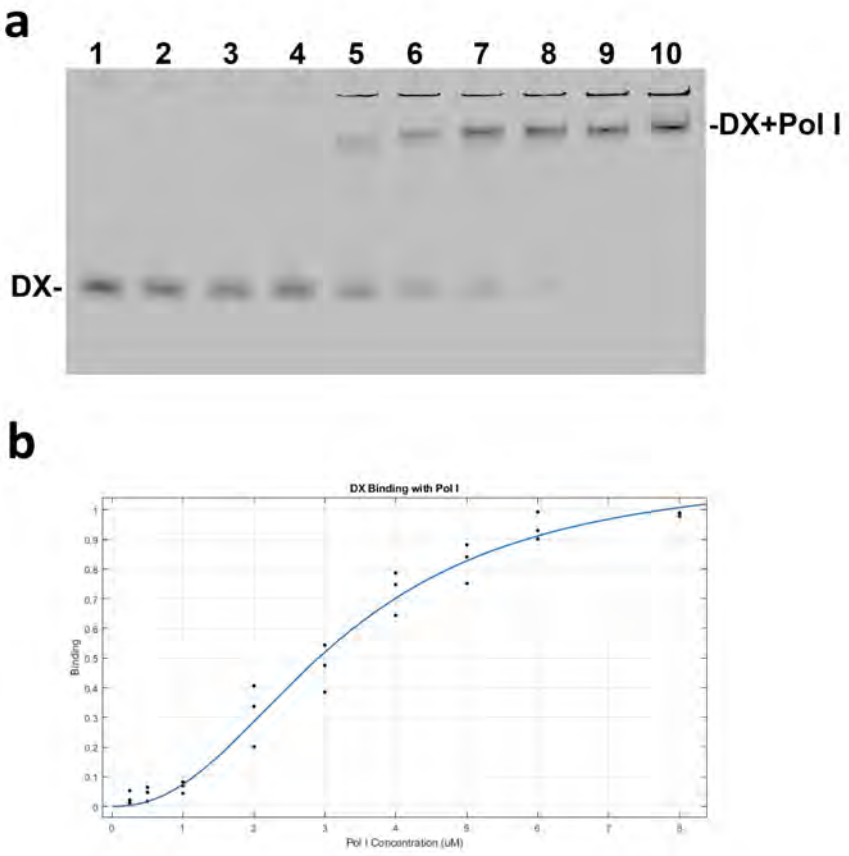


Figure S10. a) *Non-denaturing gel showing the binding results of pure Pol I to DPE DX molecule.* Lane 1 contains DX only at 2 uM. Lane 2 contains DX at 2 uM mixed with 0.25 uM of Pol I. Lane 3 contains DX at 2 uM mixed with 0.5 uM of Pol I. Lane 4 contains DX at 2 uM mixed with 1 uM of Pol I. Lane 5 contains DX at 2 uM mixed with 2 uM of Pol I. Lane 6 contains DX at 2 uM mixed with 3 uM of Pol I. Lane 7 contains DX at 2 uM mixed with 4 uM of Pol I. Lane 8 contains DX at 2 uM mixed with 5 uM of Pol I. Lane 9 contains DX at 2 uM mixed with 6 uM of Pol I. Lane 10 contains DX at 2 uM mixed with 8 uM of Pol I. b) *A graph showing the DX binding with Pol I in different Pol I concentrations*. All data from three independent experiments are shown at the graph and fitted to the model B(C) = Bmax*C^n/(C1/2+C^n), in which B(C) is the DX binding efficiency with Pol I; C is the Pol I concentration; Bmax is the maximum binding efficiency and C1/2 is the total Pol I concentration at 50% binding. According to the fitting, Bmax is 1.13; K is 14.26 uM; R-square is 0.9783 and RMSE is 0.05856. The equilibrium dissociation constant (KD) is 13.26 uM (total Pol I concentration minus bound Pol I concentration).

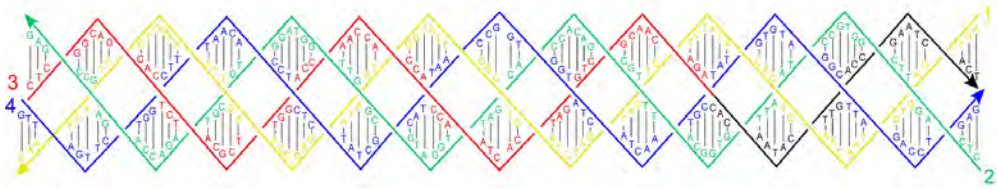


Figure S11. *The PX complex used for polymerization reaction.* To make sure that PX-DNA is stable at 37 C, we extended each of the four strands from 55 bases to 77 bases. The shorter segment removed from the complex is shown in black. If polymerization works, the shorter strand will be extended to full length.

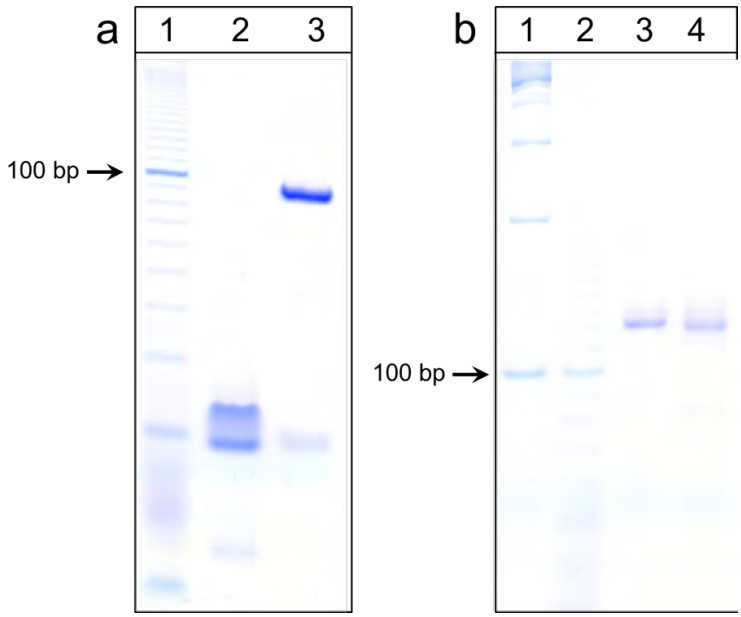


Figure S12. *Native gel of short and long PX-DNA and their substrates at 37 °C.* (a) *Short PX-DNA and its substrates at 37 °C.* Lane 1 is 10-bp marker. Lane 2 is the PX complex with a part of the red strand removed, and is not stable at 37°C. Lane 3 is short PX-DNA (each strand 55 nts long), which is still stable at 37 °C. (b) *Long PX-DNA and its substrates at 37 °C.* Lane 1 is 100-bp marker. Lane 2 is 10-bp marker. Lane 3 is long PX-DNA, which is stable at 37°C. Lane 4 is long PX substrates, with one of the strands truncated, and unlike short PX substrates, is stable at 37 °C.

Table S1. Mass spectrum scores for the isolated and purified protein fractions.

Protein Type	GN code	Score
DNA topoisomerase IV subunit A	parC	215.16
DNA polymerase I	polA	201.78
DNA-directed RNA polymerase	T7-RNA-Pol	96.72
KdgR transcriptional repressor	kdgR	78.17
DNA-binding transcriptional dual regulator	oxyR	77.76
AgaR transcriptional repressor	agaR	42.83
Lac repressor	lacI	38.92
DNA-binding transcriptional repressor	galR	32.84
Methylated adenine and cytosine restriction protein	mrr	25.98
Transcriptional regulator NanR	nanR	25.91
Predicted DNA-binding transcriptional regulator	yfeR	22.32
Global DNA-binding transcriptional dual regulator H-NS	hns	19.92
DNA gyrase subunit B	gyrB	19.28
DNA mismatch repair protein MutS	mutS	19.04
DNA topoisomerase	topA	17.54
DNA-binding transcriptional dual regulator, leucine-binding	lrp	12.34
Single-stranded DNA-binding protein	ssb	11.18
Predicted DNA-binding transcriptional regulator	yheO	9.53
DNA helicase II	uvrD	8.81
DNA-binding transcriptional activator	pspF	7.12
DNA-binding transcriptional repressor	deoR	6.28
DNA-binding transcriptional dual regulator	dsdC	5.25
AllR transcriptional repressor	allR	5.21
DNA-binding transcriptional repressor	dgsA	5.12